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# STUDIES IN THE DEVELOPMENT OF IMPROVED STRAINS OF PARASITES OF FOREST INSECTS

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## Summary

In biological control of pest insects by mean of entomophageous insects it is very important to have strains of parasites with high biotic activity.

One of the ways to obtain such strains is to explore in the field the strains of parasite having higher than average biotic activity. Then most promising races collected in various areas are crossed to receive progeny with increased biotic activity.

Such possibilities are studied in case of Dahlbominus fuscipennis Zett. and Dirhicnus alboannulatus Ratz., two important parasitic hymenopterans attacking several forest insect pests.

Collections of specimens of these two hymenopterans in different regions have been made and individuals from a given regions have been mated with individuals of another regions. Selected strains have showed improved capacity for searching out their hosts, had higher fecundity, and greater ability to survive, [redacted] will be liberated in forested areas and they will be studied to find proper techniques and conditions for their liberation.

Attempts to receive improved strains of various parasitic insects were frequently undertaken in past / G r o f f and H o w a r d , 1924; F l a n d e r s , 1930; R u b c o w , 1948/ and some of them were positive. For example, very distinct increase in efficacy of parasite as result of crossing of several strains collected in different areas of Europe, was observed in case of parasitic tachinid Compsilura concinnata Meig. introducted to the U.S.A. / H o w a r d and F i s k e , 1911/.

In the first phase of this studies on D. fuscipennis and D. alboannulatus the biology and ecology of these hymenopterans have been worked out. Informations gained in these studies served as basis to undertake further study in order to work out the most effective and economic method of laboratory mass rearing of these parasites. The method that has been developed comprises: optimal temperature - 25°C; use of most suitable insect hosts /for D. fuscipennis - Gilpinia frutitorum G., and for D. alboannulatus - Bupalus piniarius L./; storage of parasites in larval instar or in pupa in temperature 0°C., this method allows in practice to receive at any needed date the desired number of adults of both species.

In the second phase of this work studies have been made to determine the level of biotic activity of several pure lines that had been collected in different areas or lines received as result of crossing procedure. As indicators of biotic activity the following characters have been considered:

1. Longevity of females
2. Fecundity of females

3. Ability to parasitize thick-walled cocoons of hosts
4. Searching capacity of females

During the reproted period the special interest was given to the parasite D. fuscipennis. Work on second species D. alboannulatus is in the beginning stage because at time when this studies have been started /March 1961/ no sufficient number of specimens could be collected and it was necessary to wait with collection until winter, 1961-62.

[redacted].

The biotic activity of 10 pure strains of D. fuscipennis have been studied and at present time the detailed studies on several strains received from crossing of these pure lines are in progress.

Preliminary summarizing of results of studies concerning activity of investigated strains possible only at pure lines as this was studied on 16,000 individuals. As cross-lines are concerned it may be only stated that all strains of D. fuscipennis as well as D. alboannulatus, may be cross-fertilized and their progeny is fertile.

Preliminary studies on pure lines point out that there is a distinct differentiation of biotic activity among various strains of parasites and therefore special selection of strains of parasites that are used in biological control of pests is necessary. It is important to notice that as a rule the strains with high fertility and great capacity of searching of a host, showed lower ability to parasitize thick-walled cocoons and shorter average longevity or vice versa.

The practical conclusion is such that by crossing of lines with the positive features will be possible to receive a strain with ideal features for use in biological control.

## Detailed Report

### Introduction

Obtaining of effective parasites with high biotic activity is possible by elimination of most active strains collected in the field or by mean of crossing of most promising lines.

In this work, which was undertaken in order to obtain the most effective strain of two parasitic hymenopterans Dahlbominus fuscipennis Zett. and Dirhicnus alboannulatus Ratz., these two ways are applied. The received strains will be later checked in the forest as to their effectiveness in controlling some forest pest insects.

In the first phase of this studies selection of the most active strains collected in the field were made and then individuals of these lines were crossed under laboratory conditions.

Similar studies carried in different countries gave promising results. For example several papers are known reporting that crossing of strains of coccinellids collected in various areas have increased fertility of females and ability of larvae and adults to destroy aphids / J a c h m o n t o w, 1957/.

The most effective strain of Aphelinus mali Hald. has been received by selection and cross-fertilizing between three different lines of this parasite /T i l l y a r d , 1924/.

J a s u m a t s u /1958/ was succesful in finding extremely active strain of Anicetus beneficus Ishii et Jasum., that after artificial dispersion in Japan has controlled insect pest Ceroplastes rubens Mosck. All previous attempts to acclimatize other natural enemies of this pest has failed.

Very interesting results were also received during studies on Dahlbominus fuscipennis Zett. / W i l k e s , 1942, 1947/. While rearing strains of D. fuscipennis showing preferency to low temperature, in 4th generation 40% of individuals showed preferency to temperature below 10°C. In the original strain number of such individuals ranged about 3%. W i l k e s' works indicates also, that by selection of individuals that showed higher fertility was possible to increase the average fertility of females of D. fuscipennis from 34 to 68 eggs. Investigation of breeding improved strains of entomophagous insect through selection were carried by several authors and results of them were positive / B a c h de, 1958, S i m m o n d s 1947/. Therefore we see that this way show good promise in breeding of parasites for biological control purposes.

## E x p e r i m e n t a l p r o c e d u r e s

In order to obtain insect material of D. fuscipennis and D. alboannulatus the cocoons of Diprion spp. and Gilpinia spp. and pupae of Bupalus piniarius L. and Panolis flammea Schiff. were collected in different areas of Poland during autumn, winter and spring.

The pupae and cocoons were dissected in order to find parasites.

Obtained in this way strains of parasites are reared in pure lines for 3 generation at temperature 15-21°C and 50%-80% relative humidity in glass vials that are 15 mm in diameter and 120 mm in length. Each vial contained 5 females of parasites and 15 cocoons or pupae of insect host. Then experimental studies were undertaken to determine the biotic activity of separate lines of parasites.

Host insect for experimental studies of both parasites were collected in the forest areas in which appearance of these insects was reported by forest administration. In the reported period total of several thousands of cocoons and

pupae were collected. Collected diprionids cocoons were divided into two groups: /1/ - light yellow cocoons with thin wall; /2/ - black-brown cocoons with thick wall. In course of experimental studies it was found that parasitization of thin walled cocoons was 71% higher than thick walled cocoons, which were obviously more resistant against parasites' attack. First group of thin-walled cocoons included about 90% of cocoons of Gilpinia frutetorum F., while second group included 88% of Diprion pini L.

Cocoons were segregated before they were used for rearing of parasites. Cocoons damaged or with dull color /as rule killed by microorganism/ were eliminated. At further segregation a special method was used based on differences in elasticity of healthy and abnormal cocoons. It was found that normal cocoons dropped from a height of 20-30 cm on a surface of a hard wood are not repelled while dead cocoons are. Using these two methods for segregation it was possible to obtain material with health ranging about 76-82%. Segregated cocoons when stored at 0°C did not loose their vitality even for a year.

As host for D. alboannulatus the pupae of B. piniarius were used. Pupae collected in the field were segregated and abnormal pupae /which did not show movement when pressed with fingers/ were eliminated. This method allowed to obtain material 100% healthy.

The following criterions and methods were used to study biotic activity of separate strains of parasites:

1/ Longevity of females of D. fuscipennis and D. alboannulatus. Females were closed in vials /15 mm in diameter and 160 mm in length/ and kept in thermostates at 25°C and 50% relative humidity of air. Number of dead individuals was checked every day and eliminated.

2/ Fecundity of females of D. fuscipennis and D. alboannulatus. A number of tests were carried and each series included 5 one day old females and 15 cocoons or pupae of host insect closed together in a vial /15 mm in diameter and 120 mm in length/. The vials were kept in termostates at 25°C and 50% relative humidity and progeny of females were counted.

3/ Searching capacity of females. A number of tests were carried comprising separate repetitions in which insects were kept in wood cages /25 x 30 x 33 cm/ with one glass wall. In case of D. fuscipennis one repetition included 45 females and 15 cocoons, the last covered with 1 cm layer of dry forest litter. After a period of 15 days number of parasitized cocoons estimated by dissection of cocoons. In case of D. alboannulatus in similar cages 15 pupae of B. piniarius were placed on forest litter and then 45 females of parasite were closed there. Number of parasitized pupae were counted after 21 days. Because of unvailability of large termostates to keep these cages they were kept in room at temperature 15-21°C and 50-80% relative humidity.

4/ Ability to parasitize hosts with thick-walled cocoons by D. fuscipennis. This included series of tests in which each repetition comprised 20 females of parasite and 20 thick-walled cocoons placed together in a glass vial /15 mm in diameter and 160 mm long/. These vials are kept at 25°C and 50% relative humidity and number of parasitized cocoons was estimated 2 weeks later by dissection of cocoons.

Material that is used in cross breeding of pure lines is obtained by dissecting of parasitized pupae or cocoons of host insects and collecting of male and female pupae of parasites. Adults that emerge from pupae of separate lines are cross-mated and biotic activity of their progeny is checked according to the above procedure.

The studies included first generation /$F_1$/ and third generation of cross-fertilized strains because males of D. fuscipennis which hatch from unfertilized eggs /arrhenotoky/ inherit only features from mothers and therefore full appearance of results of crossing may be expected in the third generation.

## Results

It should be emphasized that because of unsufficient number of repetition of separate experiments it is to early to make complete conclusions. During the reported period special interest was given to the D. fuscipennis. Work on second species D. alboannulatus is in the begining stage as at time when this studies were started this parasite was not available in sufficient number. Collection of D. alboannulatus was possible just now in winter 1961/62.

Studies made so far on pure lines of D. fuscipennis showed that there is distinct differentiation of biotic activity in different strains /Table 1/.

Another problem studied was biotic activity of progeny received as result of cross-mating of individuals of several pure lines of D. fuscipennis. In the first phase the first generation /$F_1$/ of 18 crossed lines was studied. It was found that progeny of all strains is fertile that average longevity of females of $F_1$ /Table 2/ was higher comparing with females of pure lines /Table 1/.

In the second phase that is in the begining stage special attention was given for combination of these pure lines which have showed special positive features. The $F_1$ and $F_3$ were studied and results are presented in Table 3.

As regards D. alboannulatus 7 pure strains were collected in various areas and rearing stocks were established. It may be emphasized that there are some difficulties in rearing of parasites connected with appearance of unexpected diapause of larvae and complete lack of males in some population. This last leads sometimes to extermination of strain because from unfertilized eggs hatch only males.

Table 1

| Strain Number | Longevity of females | | Parasitization of thick-walled cocoons | | Searching capacity | | Fecundity | |
|---|---|---|---|---|---|---|---|---|
| | Number of females | Average longevity in days | % parasitized | Number of cocoons tested | % parasitized | Number of cocoons | Average fecundity of one female | Number of females tested |
| I. | 828 | 7,9 | 38,2 | 300 | 56,3 | 225 | 47,5 | 305 |
| II. | 819 | 6,1 | 23,8 | 285 | 67,7 | 225 | 46,0 | 300 |
| III. | 794 | 7,8 | 41,8 | 300 | 71,7 | 225 | 35,3 | 295 |
| IV. | 824 | 7,2 | 36,0 | 300 | 50,0 | 225 | 37,0 | 300 |
| V. | 545 | 7,4 | 31,5 | 300 | 47,0 | 225 | 45,6 | 295 |
| VI. | 816 | 6,8 | 22,8 | 300 | 54,0 | 225 | 52,1 | 300 |
| VII. | 812 | 7,0 | 30,3 | 300 | 45,2 | 225 | 39,4 | 305 |
| VIII. | 824 | 6,7 | 23,7 | 300 | 41,7 | 225 | 48,9 | 300 |
| IX. | 823 | 6,7 | 27,8 | 300 | 43,8 | 225 | 55,0 | 305 |
| X. | 809 | 6,6 | 33,8 | 300 | 53,8 | 225 | 46,1 | 255 |

Table 2

| Number of cross-mated strains | Longevity of females | | Remarks |
|---|---|---|---|
| | Average longevity /in days/ | Number of females tested | |
| I x II | 9,1 | 404 | |
| II x VI | 8,6 | 216 | |
| I x III | 8,2 | 429 | |
| II x VII | 8,0 | 138 | |
| II x III | 8,3 | 210 | |
| I x IV | 8,4 | 221 | |
| I x V | 10,6 | 196 | |
| V x VII | 9,2 | 237 | |
| II x V | 9,1 | 224 | |
| I x VI | 8,2 | 308 | |
| II x IV | 8,6 | 212 | |
| III x V | 9,3 | 219 | |
| V x VI | 8,3 | 225 | |
| IV x VII | 9,2 | 164 | |
| III x VI | 8,5 | 400 | |
| VI x IX | 7,2 | 391 | |
| III x IX | 7,5 | 417 | |
| I x IX | 7,6 | 242 | |

Table 3

| Number of cross-mated strains | Longevity of females | | | | Fecundity of females | | | |
|---|---|---|---|---|---|---|---|---|
| | $F_1$ | | $F_3$ | | $F_1$ | | $F_3$ | |
| | Average longevity in days | Number of females tested | Average longevity in days | Number of females tested | Average fecundity | Number of females tested | Average fecundity | Number of females tested |
| IxII | 9,1 | 404 | - | - | 55,1 | 100 | - | - |
| IxIII | 8,2 | 429 | 7,8 | 140 | 54,5 | 150 | 57,4 | 50 |
| IxVI | 8,2 | 308 | 7,9 | 200 | 47,0 | 75 | 54,3 | 65 |
| IIIxVI | 8,5 | 400 | 7,6 | 216 | 59,1 | 115 | 40,8 | 75 |
| IxIX | 7,6 | 242 | 7,6 | 242 | 49,6 | 125 | 58,4 | 65 |
| IIIxIX | 7,5 | 417 | 7,3 | 328 | 54,5 | 85 | 55,6 | 110 |
| VIxIX | 7,2 | 391 | 7,6 | 200 | 58,7 | 150 | 76,3 | 50 |

Table 3 like Table 2 also shows that vitality of progeny of crossed lines increases in $F_1$ and $F_3$. However, because of unsufficient number of repetitions it is difficult to say now whether crossing of most prominent strains will give progeny with high vitality.

Several experiments were carried to find what host insects appear to be the best for laboratory rearing of parasites. Out of several natural insect hosts: Panolis flammea, Sphinx pinastri and Bupalus piniarius or unatural /for the first time used as hosts for D. alboannulatus/ - Galleria mellonella L., Malacosoma neustria L., and Ephestia kuhniella Zell. as best host appeared to be B. piniarius. This insect is quite easy to obtain in great number in forest and pupae are easily stored for a long time at 0°C without loosing their vitality. Therefore, few thousands of pupae of B. piniarius were collected in the field and they will be used in the laboratory rearing of D. alboannulatus.

Some points in biology of D. alboannulatus have been explained /this is used as basis for studies on biotic activity of this parasite/:

1/ average quantitative relation between females and males is 9:1 /2000 individuals were checked/,

2/ average fecundity of females at optimal conditions is about 175 eggs /500 females were checked/,

3/ length of development of one generation is 20,5 days /at temperature 25°C and 50-60% of relative humidity of air/,

4/ when temperature during rearing of parasites decreases below 14°C a great percent of larvae undergo diapause for a few months.

## Discussion

Results of studies made up to now upon development of improved strains of parasites D. fuscipennis and D. alboannulatus are not yet complete and collecting of additional observations is required.

However, on the basis of obtained results it may be stated the separate strains of studies parasites collected in various areas differ significantly in biotic activity. Therefore it is very important to use for intraareal introduction or acclimatization of new parasites only strains with biotic activity higher than average. It may be emphasized that although in the literature decrease of biotic activity of pure lines inbreeded for a long time is mentioned / R u b c o w, 1948/ in this study this phenomenon was not observed. Because crossing between all strains of D. fuscipennis and D. alboannulatus is feasible the geographic races are not easily formed as it is observed at some parasites / T i e l e n g a, 1956, 1959/.

As regards the possibility of improving of collected in field strains by mean of cross-mating of pure lines it is to early to make final conclusions. However, preliminary observations on crossing of D. fuscipennis showed that biotic activity of progeny has increased. Whether this phenomen will disappear after 3-4 generation e.g. like effect of heterozy observed in case of many entomophagous insects / R u b c o w 1948, J a c h o n t o w 1957/ or this is inherited feature it will be found in the future investigations.

It should be pointed here that some difficulties may arise in further studies on improving of strains of D. fuscipennis and D. alboannulatus by mean of cross-mating of most positive pure lines connected with fact that e.g. strains showing high fertility or ability to parasitize thick walled cocoons have low capacity of searching of hosts and low longevity or vice versa.

Therefore it may be assumed that only by further careful selection of progeny of crossed most positive strains in respect of biotic activity it will be possible to obtain pure lines of parasites having required fecundity, searching capacity of hosts and ability to parasitize thick walled cocoons.

## Conclusions

Studies made so far have showed that in laboratory rearing is it possible to obtain any required number of individuals of both studied species at any date. This finding is of great importance for further studies on mass introduction of most vital strains of parasites, received by selection

and crossing, into forest areas to check their efficacy in controlling insect pests in field.

It may be emphasized that long lasting rearing of parasites has no negative influence on vitality and activity of studied strains. All studied strains of parasites collected in various parts of Poland differ in respect to their biotic activity. The lowest differences observed concerned the average longevity of females.

The most positive strains in one respect e.g. fecundity showed inferiority in other respect e.g. longevity of females.

Preliminary studies upon cross-mating of individuals of different strains has indicated that crossing is feasible and that it is possible to increase biotic activity of parasites by this method. This last conclusion must be, however, verified by conducting additional studies.

In the present stage of studies special interest must be given for crossing of all positive pure lines and received progeny should be selected to obtain strain or strains of parasites having high vitality and activity. It must be also cleared experimentally by laboratory rearing of few generations of parasites, whether such positive features will be inherited or shall disappear as temporary effect of heterozy. Obtaining of strains with permanent features consolidated by selection will have great value for biological control purposes.

## Plan of future work

The plan for the second year of our investigation includes completing and starting of the following problems:

A/ At Dahlbominus fuscipennis

1. Completing of studies on biotic activity of pure strains of parasite in order of definite finding what strains are most effective.

2. Continuation of crossing of most active strains. Special interest will be given to crossing of some most positive pure strains and selecting from mixed populations the pure lines having all required positive features.

3. Collecting of few thousands of cocoons of Diprion spp. and Gilpinia spp. for further laboratory rearing of parasites.

4. Obtaining in laboratory rearing of several thousands of individuals that will be used for introduction and checking of their efficacy under natural conditions.

B/ At Dirhicnus alboannulatus

1. Collecting of additional pure strains of parasite from various areas.

2. Studies on biotic activity of pure lines.

3. Crossing between most active strains.

4/ Collecting of few thousands of pupae of Bupalus piniarius for further propagation of parasite.

5/ Obtaining in laboratory rearing of several thousands of individuals that will be used for introduction and checking of efficacy of this parasite under natural conditions.

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